a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/cholesterol bilayer. Twenty-five independent system setups, each containing sixteen equally spaced and randomly oriented parallel receptors, were simulated for 10 microseconds each, to investigate spontaneous self-assembly of DOP, MOP, or KOP homomers, as well as DOP-MOP and DOP-KOP heteromers, using the MARTINI coarse-grained force field. From these 250 microseconds of simulation we have identified several common dimeric arrangements, predominantly involving specific combinations of transmembrane helices TM1, TM2, TM4, TM5 and TM6, and the juxtamembrane helix 8. Unlike the TM5/TM6 and TM1/TM2/H8 interfaces observed in the MOP crystal structure, the TM1/TM2/H8 interface presented by the KOP crystal structure was reliably reproduced during spontaneous association of the KOP, DOP, and DOP-KOP dimers within the lipid environment. MOP dimers did reproduce other crystallographic dimers sporadically, namely the TM1/TM2/H8 and TM5/TM6 interfaces from the beta2-adrenergic receptor and CXCR4 crystal structures, respectively. These extensive simulation data provide testable hypotheses of likely homo- and hetero-dimerization interfaces, as well as diffusion rates, for all major opioid receptor subtypes, contributing important insights into the nature of association of these receptors in a lipid membrane environment.

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All-Atom Simulations Reveal Ensemble Dynamics of Rhodopsin

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G protein-coupled receptors (GPCRs) are a biomedically important class of integral membrane proteins that function by transducing signal across the cell membrane. They work as allosteric machines, with structural changes cascading through the protein to modulate activity, a mechanism that is not fully understood. Rhodopsin, the mammalian dim-light receptor, is a model GPCR that provides a unique test case for understanding allostery. The ligand-bound protein acts as a two-state switch with minimal basal activity. However, its apo-form (opsin) is outside the normal activation cycle and may behave differently. Structural data show opsin in an active-like state, but physiologically it has only minimal activity. Here, we explore opsin's ability to fluctuate between states and test the ligand's role in activation. We performed an ensemble of microsecond-scale simulations (~60 microseconds in all) using four systems: two with the ligand present and two without. Opsin fluctuated between states, showing that both active-like and inactive-like structures may be part of its conformational ensemble. To further study the structural ensemble available to these systems we applied clustering methods. We found that the ligand-free trajectories are better able to sample both inactive-like and active-like conformations but that the four systems are converging. The underlying process is clearly not as simple as a lock and key mechanism.

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Identification of an Endogenous Allosteric Modulator's Binding Site at the Human Cannabinoid-1 Receptor, using the Forced-Biased Metropolis Monte Carlo Simulated Annealing Method (Mmc)

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The CB_1 endogenous, positive allosteric modulator, lipoxin A_4, increases the equilibrium binding and efficacy of CP55,940 and anandamide (orthosteric agonists), yet has no significant effect when applied alone. Unlike ORG27569 (a negative allosteric modulator of CB_1), lipoxin A_4 does not significantly displace SR141716A (an orthosteric inverse agonist) in equilibrium binding assays. In addition, we have previously reported that ORG27569's binds in the THM3/TMH6/TMH7 region (Shore et al., *ICRS*, **2012**); at this site, ORG27569 sterically blocks important movements of the second and third extracellular loops, as well as those of TMH6, that are necessary for G protein-mediated signaling. Because lipoxin A_4 is a positive allosteric modulator, one would not expect it to sterically block these functionally-important conformational changes. Together, these results may suggest that lipoxin A_4 binds in a topographically different region of CB_1 than ORG27569.

To identify lipoxin A_4's binding site(s) at CB_1, we are using the Forced-Biased Metropolis Monte Carlo simulated annealing program, MMC. In this method, lipoxin A_4 was separated into 4 fragments. Four MMC runs are currently being performed, in which our *in silico* CB_1 receptor model (with CP55,940 docked) was immersed in a box filled with copies of one of these fragments. The system chemical potential is then systematically annealed, causing only those fragment copies with the best free energy of binding to the protein surface to remain. Ultimately, analysis of these four MMC runs will suggest region(s) in which all four fragments cluster in the correct spatial proximity, thus suggesting a possible binding site(s). [Support: RO1 DA003934 and KO5 DA021358 (PHR)]

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Highly Enhanced Conformational Sampling of the Transmembrane Domain of Egf Receptor Sheds Light on the Activation Mechanism

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The epidermal growth factor (EGF) receptor plays critical roles in regulating proliferation, survival and migration of cells. The activation of this receptor tyrosine kinase involves the dimerization of alpha helices in the transmembrane (TM) domain. Single point mutations in this region can modulate efficiency of helix dimerization and abnormal activation leads to human cancers. Therefore, it is crucial to determine modes and affinities of association in functional receptors and oncogenic forms. Various experimental and computational techniques have been recently used in this respect, which have provided substantial results. The emergence of innovative approaches might strongly contribute to improve our understanding of the activation mechanisms.

Here, we develop a new computational method that combines coarse-grained molecular dynamics and metadynamics to investigate the dimerization of TM helices of EGF receptor. We perform simulations above the millisecond timescale to explore multiple events of association and dissociation. Our enhanced sampling protocol enables reconstruction of the multidimensional free energy landscape of the TM domain in its lipid bilayer environment. The results reveal complex free energy surfaces on which pathways of dimerization are clearly located. Then, cluster analyses enable identification of dimer conformations with N-terminal contacts of marginally higher stability than conformations displaying C-terminal contacts. Overall, the results suggest a mechanism in which the TM domain is a soft pivot and the dynamics of both ectodomain and juxta-membrane domain would drive the conformational change from inactive to active states of EGF receptor dimers. Application of the method to the homolog receptor Her2 will be also discussed to illustrate potential differences in the mechanisms of activation.

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Dimerization of Transmembrane Helices of Receptor Tyrosine Kinases: Assessing the Convergence of Coarse-Grained Molecular Dynamics Simulations

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Signal transduction is essential for many biological processes, such as mitosis, cell regeneration and differentiation. The transmembrane (TM) domains of many signalling proteins are known to play an essential part in these processes, focussing structural and biophysical studies on transmembrane helix dimers. This is especially true of the receptor tyrosine kinase (RTK) family, for which many mutations are implicated in diseases including e.g. breast cancer, myasthenia gravis, and achondroplasia. Structures of a number of transmembrane helix dimers have been solved using solution NMR applied to peptide/detergent complexes, but many of these transmembrane helix dimers remain uncharacterised. A multiscale Molecular Dynamics (MD) simulation approach can yield models of comparative accuracy to NMR structures, making it a valuable tool to use for systems where the helix dimer structure is still unknown. The challenge then arises in determining the number of courage-grained (CG-MD) simulations needed to converge upon representative structures of the dimer, which can then be refined by converting to atomistic representation and simulated to compare with potential NMR structures. Here, an iterative jackknife approach is used to assess the convergence of CG-MD simulations, using the well-characterized helix dimer system of ErbB1. This approach will be illustrated for the transmembrane helix dimer of the RTK Muscle-Specific Kinase, whose transmembrane helix remains uncharacterised.

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Association of Epha2 Receptor with the Membrane through its Second Fibronectin Domain: A Biophysical and Computational Study

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Eph receptors are cell surface type I transmembrane receptors involved in numerous cellular processes. Ligand-binding causes Eph clustering, a process that is, to a large extent, driven by the extracellular region of the receptor. The structural mechanisms that underly Eph clustering have recently been elucidated by crystal structure data for complete Eph ectodomains and their ligands. While it is understood that the clustering depends largely on the N-terminal domains of the extracellular region, little is known about the functions of